

# Biostimulation of pesticide degradation in groundwater aquifers with addition of oxygen at different concentrations

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These primers were used to monitor the evolution of PHA-storing microorganisms during the enrichment step (qPCR), but they also allowed determining PHA synthesis activity during the accumulation step (RT-qPCR).

Two sequencing batch reactors (SBRs) were inoculated with activated sludge and operated under FF conditions (operating parameters consistent with the current state of the art for mixed culture PHA production). Two distinct feeding regimes were imposed. The impact of the distinct operating conditions on the microbial population dynamics were followed assessing microbial diversity (DGGE) and function specialization-enrichment in PHA storing organisms (qPCR) and activity of the PHA storage function (RT-qPCR).

The developed molecular tools allowed describing a dynamic of the bacterial population specific to the feeding strategies imposed. A simplification of the bacterial populations toward dominant PHA producers was more distinctly observed for one of the two cases. Moreover, these populations seem to have a specific activity response depending on the feeding rate.

**429B Biostimulation of pesticide degradation in groundwater aquifers with addition of oxygen at different concentrations**

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Pesticides are detected in an increasing number of aquifers all over the world resulting in closure of many water abstraction wells. The natural redox conditions of aquifers are considered to be an important factor governing the pesticide degradation in ground water. These observations emphasize the importance of the only possible process mineralization as a result of the biological processes to complete removal of pesticides from the environment. Microbial degradation of organic pollutants in groundwater takes place naturally, sustained by electron acceptors, electron donors and nutrients or using enhanced bioremediation strategies. One relevant approach for the remediation of pesticides in contaminated aquifers is to stimulate indigenous microbial populations with addition of electron acceptors such as oxygen. In this research we investigate the ability and effects of oxygen addition at different concentrations to stimulate biodegradation of (RS)-2-(4-chloro-2-methylphenoxy) propanoic acid (mecoprop), (R)-2-(2,4-dichlorophenoxy) propanoic acid (dichlorprop) and 3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide (bentazone) in an anaerobic sediment and groundwater.

Anaerobic sediment and groundwater samples were collected from 2.3 to 7.1 mbs from a Danish aquifer. Experiments were conducted with groundwater and sediment in laboratory batch systems amended with oxygen concentrations 0.0, 0.1, 0.5, 1, 2, 4, 5, 8, 9 and 11 mg $L^{-1}$. The microcosms were incubated in the dark at 10°C for 200 days. $^{14}C$ labeled mecoprop, dichlorprop and bentazone mineralization were monitored as produced $^{14}CO_2$. Oxygen concentrations were measured from outside of the microcosms by a fiber optical oxygen meter using oxygen sensitive luminescent sensor foil mounted inside the microcosms at the beginning of set up and during the sampling periods. More oxygen was added during the experiment to achieve the desired oxygen concentration level in each bottle.

The results demonstrate that addition of oxygen has a positive effect on herbicides degradation. The highest mineralization potential was found in the microcosms amended with high oxygen concentrations. Here, 14-27 % of mecoprop, 3-9 % of dichlorprop and 15-20 % of bentazone were degraded over 200 incubation days. There was a clear increase in mecoprop and bentazone degradation in highly oxygenated microcosms. Furthermore, addition of low oxygen concentration (1 mg $L^{-1}$) would be adequate to initiate and have significant impact on aerobic degradation. We have observed no mineralization in the control microcosms that were inactivated microbiologically or microcosms that were not amended with oxygen, indicating an occurrence of the microbial degradation and that need of oxygen for biodegradation of the herbicides. To our knowledge, this is one of the first reports showing a link between the oxygen addition and bentazone degradation in aquifer systems.

Our findings suggest that shifting anaerobic conditions to aerobic conditions by increasing oxygen concentration would provide a potential and promising remediation technology for enhancement of

microbial degradation of pesticides, although high oxygen consumption by organic matter and reduced species may limit the application of enhanced bioremediation.

**430B T-RFLP and Pyrosequencing Approach to Clarify Bacterial Groups that Affects Dewaterability of Activated Sludge**

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Bacteria which affect dewaterability of activated sludge were investigated in this study. In Japan, waste biomass generated from biological wastewater treatment is often dewatered and then incinerated. The performance of dewatering affects the energy efficiency of incineration process, and thus the control of dewaterability is the key in waste biomass treatment. However, factors that affect dewaterability of waste biomass are not well clarified yet. In the present study, we hypothesized that certain group of bacteria is positively or negatively affecting dewaterability of waste biomass. We operated a laboratory activated sludge reactor for 214 days with synthetic wastewater, and monitored dewaterability of activated sludge biomass and microbial population at 73 sampling days. Dewaterability was monitored by water content of dewatered (WCDS) testing method. Microbial population was analyzed by molecular methods targetted at a partial 16S rRNA amplified by a 27f/519r primer set: Terminal Restriction Fragment Length Polymorphism (T-RFLP) was conducted with a 27f primer labeled with FAM and RsaI restriction enzyme, and pyrosequencing was done with barcoded 27f and 519r primers. PCR products were sequenced from forward or reverse primer ends by a Roche 454 FLX+ pyrosequencer. Obtained sequences were assigned to their original samples, restriction sites in reads with forward primer region were identified, and reads count for different "virtual" terminal restriction fragment sizes were calculated. In T-RFLP analysis, three of the terminal restriction fragments (T-RFs) and four T-RFs were found to have positive and negative correlations with dewatrability, respectively. Multiple regression analysis of these 7 T-RFs with dewaterability showed a high correlation with R^2=0.84. By pyrosequencing, a total of 78761 reads were obtained, or per sample, 1079 reads in average ranging from a minimum of 325 (Day 65) to a maximum of 8217 (Day 163) reads. The number of reads which had forward primer region and effectively used for virtual T-RFLP was 69343. Each T-RF size contained fragments from plural OTUs. Yet, major OTUs related to each T-RF were identified. Following bacterial groups were suggested to be associated with the 3 T-RFs that had positive correlation with dewaterability: Myxococcales (closest to Haliangiaceae with 49% confidence), Planctomycetales, Thiothrix at genus level respectively. The 4 T-RFs negatively related with dewaterability were associated with following groups: Bacteroidetes (closest to class Ohtaekwangia with 92% confidence.), two groups in Alphaproteobacteria both closest to Rhizobiales at confidence levels of 97% and 40% respectivley, Haliscomenobacter and Propionivibrio at genus levels.

**431B Strategies for the management of surface microbial communities based on volatile compounds and visible light**

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In our everyday life we constantly stay in contact with surfaces. These surfaces can be compared with ecosystems, which contain microbial communities. Especially surfaces in indoor environments are directly influenced by humans and their microbes. To date disinfection techniques were applied with the objective to kill the whole microbial community including beneficial bacteria. Bacterial antagonists, who belong to this group, suppress the growth of other organisms, including pathogens, by competition and secretion of antimicrobial/lytic substances. In this context, volatile organic compounds (VOCs) show a novel mechanism for bacterial antagonism and biocontrol of pathogenic organisms. Beyond that, photobiology and the influence of light on living organisms could also be used to control microbial communities.

We analyzed endophytic bacteria isolated from mistletoe (*Viscum album*) and Styrian oil pumpkin (*Cucurbita pepo* var. *styriaca*) in dual culture assays combined with GC-MS headspace analysis for